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A Short Evaluation of a New Haematological Analyser: The Cobas Argos 5 Diff

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Summary: In order to evaluate the Cobas Argos 5 Diff, a five-part differential haematological analyser, we performed a comparative study using our current analyser, the Toa E-5000, and the manual leukocyte differential. Samples (n = 593) were collected from various departments in our hospital and were tested on both Cobas Argos 5 Diff and Toa E-5000. Regression lines were calculated for all standard haematological quantities. A group of 100 samples, including 50 negative (absence of flags) and 50 positive (presence of flags), on the Cobas Argos 5 Diff were randomly selected from the above-mentioned collection. All these samples had been checked under the microscope by two persons counting 200 cells each.

The comparison of standard haematological quantities between Cobas Argos 5 Diff and the Toa E-5000 shows good correlation: Red Blood Cells r = 0.993; White Blood Cells r = 0.998; Haemoglobin r = 0.989; Haematocrit r = 0.982; Thrombocytes r = 0.988; Lymphocytes (%) r = 0.983; Neutrophils (%) r = 0.959. The correlation between Cobas Argos 5 Diff and the manual leukocyte differential shows good correlation for lymphocytes and neutrophils, with acceptable correlation for eosinophils. The correlation for basophils and monocytes was less acceptable, especially for normal samples. We conclude that the efficiency of the Cobas Argos 5 Diff is good (93%) and that it is a suitable haematological cell analyser.

Introduction

The reference for electronic differential leukocyte analyser evaluation is the method established by the U.S. National Committee for Clinical Laboratory Standards (NCCLS) (1) named H20-T. This method uses as a reference the manual leukocyte differential performed on 150 normal and 150 abnormal specimens, counting 800 cells in each to reduce the subjective aspects of the classical 100-cell differential count.

The need for haematological analyser evaluation has led several authors to develop their own methods, which are basically adapted from the H20-T procedure, in order to be more suitable for their own laboratories in terms of specimen collection and personnel. In particular, the methodology developed by *Kohut* is quite frequently used at this time (2.3).

A classic way of evaluating a haematological analyser is to compare the performances of the new one versus another one that has proven to be reliable (4).

Such methods are applicable for comparing the basic counts, e. g.: red, white cells and platelets, as long as the counting systems are based on the same principle. This is possible today as a majority of counters operate on the aperture impedance method originally developed by Coulter.

As for the white cell differential, a comparison between two different automatic analysers is almost impossible to interpret statistically because the principles of differential may vary dramatically from one instrument to another.

In the latter case, the reference method remains the manual leukocyte differential under certain conditions described in the literature (5).

We therefore compared the basic counts from the Cobas Argos 5 Diff with ones from a current blood cell counter. The white cell differential performances were evaluated versus the manual differential.

Materials and Methods

System description

The Cobas Argos 5 Diff (manufactured by ABX, France, member of the group Hoffmann-La Roche AG) is a fully automatic blood cell counter and analyser. Whole blood (200 µl) is drawn from a closed tube, from this an aliquot of 25 µl is taken through a ceramic shear valve for performing the count of platelets, red and white blood cells. The count is performed using the variation of impedance. Haemoglobin is measured by a modified haemiglobin cyanide absorbance method.

A second aliquot of 25 µl is mixed with a specific reagent that induces red blood cell lysis and the staining of the eosinophil granules. The white cells are transferred through a chamber (ABX patent) where the volume of each cell is measured (variation of impedance), as well as its light absorption (from an halogen source). From these measurements a chart is plotted. (X axis = volume; Y axis = absorbance). The groups of cells appearing on the chart are lymphocytes, neutrophils, eosinophils and monocytes + basophil group.

A third aliquot of 15 µl whole blood is mixed with a reagent which lyses all the cells except the basophils. These are measured by variation of impedance in a separate chamber. The value obtained here is substracted from the value of the monocyte + basophil group described above.

From the print-out of the results, a sample is declared negative (normal) if no flag is present. A sample is declared positive (abnormal) if one or more flags appear.

Specimen collection

Blood samples (n = 593) were collected in Sarstedt monovettes K_2-EDTA. After mixing each sample it was divided of into two tubes to avoid the problem of different mixing. All the samples were analysed within two hours after sampling.

Comparison with the Toa-Sysmex E-5000

We compared the performance of the basic counts from the Cobas Argos 5 Diff with the same from the Toa E-5000 counter which is currently in use in our lab. The latter has proven to be reliable in blood cell counting (6).

Reference method

We selected randomly 100 samples (including 50 positive and 50 negative on the Cobas Argos 5 Diff) from the group described above and we made blood smears from them. After drying and staining the blood smears with a Shandon system according to the *May-Grünwald* method, 200 cells were counted twice by two specialists using a double blind method, under analytical criteria recommended by the NCCLS. If a major difference between the two counts appears, a third count is performed on 400 cells.

Statistical calculations

For the calculation of the regression lines we used the procedures and corresponding formula developed by *Passing & Bablok* (7, 8).

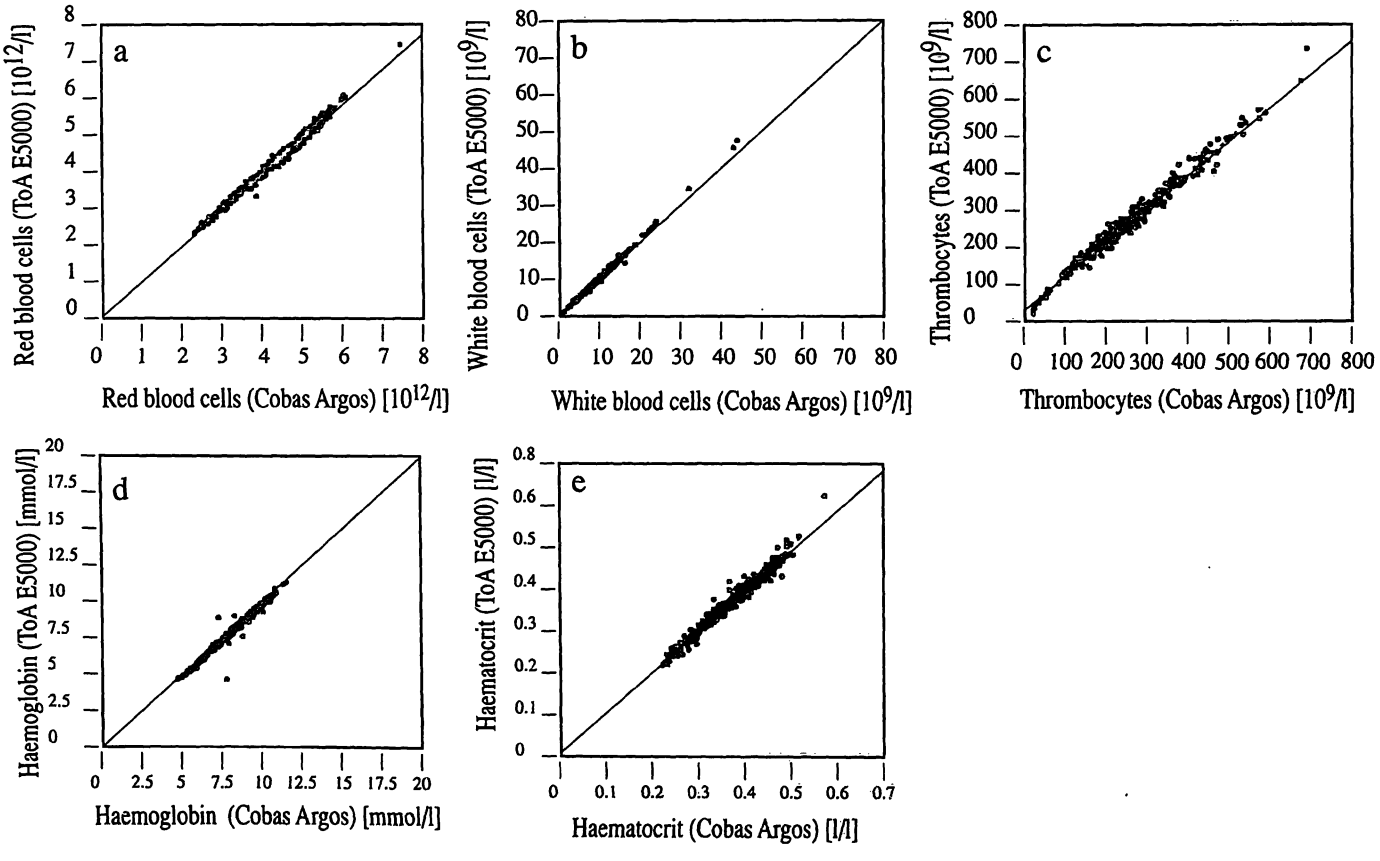

Fig. 1. Orthogonal regression of the Cobas Argos Versus the ToA E5000 N = 380

a: Red blood cells:	Intercept = −0.035,	slope = 0.975;	r = 0.993;
b: White blood cells:	Intercept = −0.2,	slope = 1.000;	r = 0.998;
c: Thrombocytes:	Intercept = −30.107,	slope = 0.929;	r = 0.988;
d: Haemoglobin:	Intercept = −0.1,	slope = 1.000;	r = 0.989;
e: Haematocrit:	Intercept = −0.007,	slope = 0.971;	r = 0.982.

The *Passing & Bablok* method for calculating the parameters of the regression line makes no special assumptions regarding the distribution of the samples and the measurement errors. The result does not depend on the assignment of the methods or instruments to X and Y. To obtain reliable results, the authors recommend assuring the sample size as well as the sampling distribution, the precision of the methods and the concentration range covered by the samples.

For the calculation we used a program developed by Boehringer Mannheim based on the *Passing & Bablok* formula.

Results

In the first part of our investigation we compared the results of the normal count of the 593 samples (red and white blood cells, haematocrit, haemoglobin, platelets) from the Cobas Argos 5 Diff with those given by our current counter the Toa System E-5000 (fig. 1).

Both analysers were calibrated following the instructions from the manufacturers.

In the second part of this study, we compared the performance of the Cobas Argos 5 Diff with that of the manual leukocyte differential.

We calculated the regression lines between Cobas Argos and the manual differential for each quantity, separately for normal and abnormal samples as defined above (figs. 2 and 3).

Sensitivity and specificity

The results were reported as true negative (TN) if the results from the analyser and the manual leukocyte differential were both negative; true positive (TP) if the results from both techniques were positive; false negative (FN) in cases of negative results given by the analyser and positive by microscope determination; false positive (FP) in cases of a positive results reported on the analyser and negative by microscope.

The assessment matrix is shown in table 1.

Different abnormalities found in the positive cases are compiled in table 2.

Classical indices have been calculated according to the following formula:

- Specificity = TN/(TN + FP) = 91%
- Sensitivity = TP/(TP + FN) = 96%

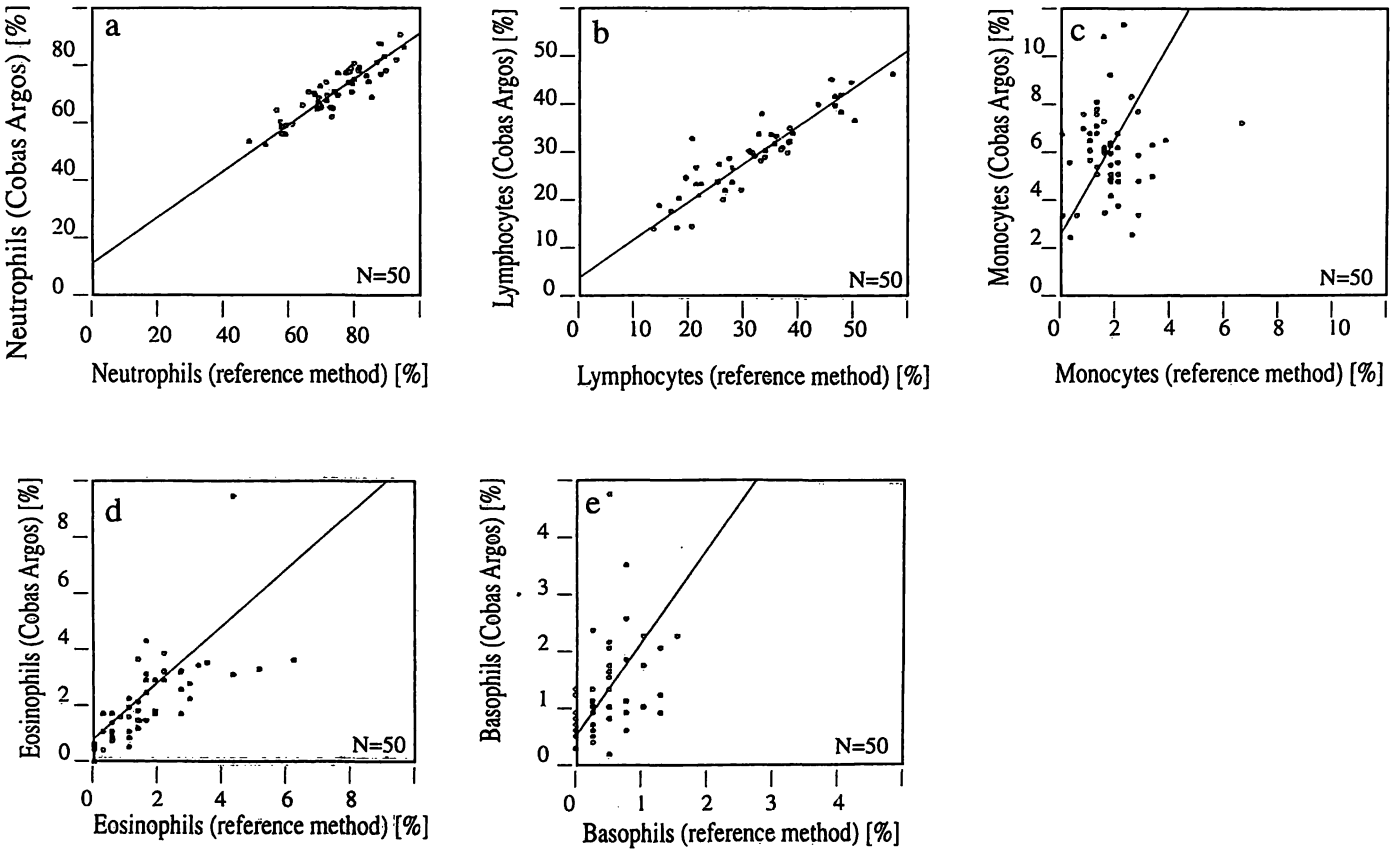

Fig. 2. Orthogonal regression of the Cobas Argos versus the 2 × 200-cell manual differentiation (Normal samples)

a:	% Neutrophils:	Intercept = +8.99,	slope = 0.812;	r = 0.902;
b:	% Lymphocytes:	Intercept = +3.7,	slope = 0.80;	r = 0.911;
c:	% Monocytes:	Intercept = +2.8,	slope = 2.0;	r = 0.099;
d:	% Eosinophils:	Intercept = +0.4,	slope = 1.025;	r = 0.699;
e:	% Basinophils:	Intercept = +0.49,	slope = 1.64;	r = 0.415.

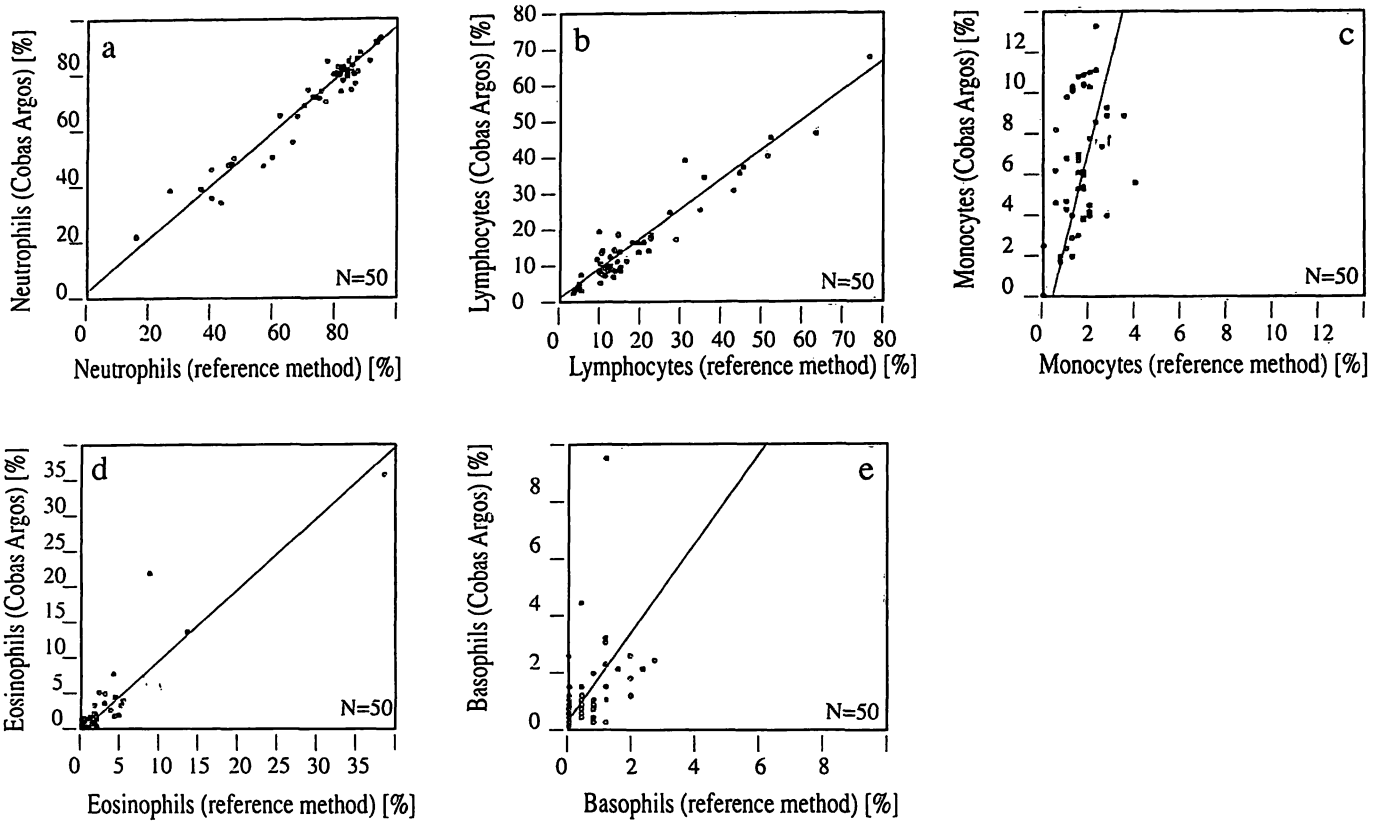

Fig. 3. Orthogonal regression of the Cobas Argos versus the 2 × 200-cell manual differentiation (Abnormal samples)
a: % Neutrophils: Intercept = +2.88, slope = 0.933; r = 0.969;
b: % Lymphocytes: Intercept = +0.90, slope = 0.821; r = 0.958;
c: % Monocytes: Intercept = −0.92, slope = 4.2; r = 0.386;
d: % Eosinophils: Intercept = −0.1, slope = 1.000; r = 0.930;
e: % Basophils: Intercept = +1.99, slope = 1.60; r = 0.401.

Tab. 1. Evaluation of the Cobas Argos 5 Diff performances versus the manual leukocyte differential.

	Cobas Argos 5 Diff	
	Negative	Positive
Negative (manual leukocyte differential)	48	5
Positive (manual leukocyte differential)	2	45

Tab. 2. Abnormalities found in the positive cases

n	Abnormalities
14	Left shift
2	Toxic granules
13	Neutrophilia
5	Eosinophilia
4	Lymphocytosis
3	Chronic lymphoid leukaemia
2	Atypical lymphocytes
2	Monocytosis
5	Red blood cell abnormalities

- Efficiency = TP + TN/(TP + TN + FP + FN) = 93%
- Predictive value of a positive result = (PV+) = TP/(TP + FP) = 90%
- Predictive value of a negative result = (PV−) = TN/(TN + FN) = 96%

On the Cobas Argos 5 Diff, 5 false positive and 2 false negative samples were found. The false negative samples on the Cobas Argos 5 Diff were reported on the manual leukocyte differential with the following abnormalities:

1) atypical lymphocytes; and
2) virally infected lymphocytes

Discussion

The Cobas Argos 5 Diff has already been compared shortly with the Technicon H2 by *W. Goossens* (9), with special emphasis on precision, linearity and carryover. Globally, in this study, the performances were found to be in agreement with manufacturer claims,

which are the usual ones expected for such types of instrument, except, perhaps, for the white blood cell linearity curve.

The aim of the present study was to compare the performance of the Cobas Argos 5 Diff on the basic counts with our current blood cell counter, the Toa Sysmex E-5000.

The Toa Sysmex E-5000 is a 3-part differential instrument which has given satisfactory performance in blood cell counting, but it is not an appropriate reference for testing a 5-part differential analyser.

Nevertheless, performance on the basic counts and haemoglobin measurement of the Cobas Argos 5 Diff was evaluated versus the Toa E-5000 by calculating the linear regression lines following the original procedure developed by *Passing & Bablok*.

These authors have developed a procedure of calculation and tested it versus the classical method of calculation. The superiority of their method has been proven by means of a simulation study and recommendations made by the authors have been carefully considered in our study: These included minimal sample size, coefficient of variation (CV) on both techniques, ranges and absolute values of the collected data.

We therefore tested 593 samples. Because the program we used to calculate the regression line accepts a maximum of 380 samples, we calculated the regression lines twice on 380 samples and 213 samples and we found the same results.

In the same way we determined the regression lines separately for both normal and abnormal results in order to increase the power of the statistical test by decreasing the spread of the results.

The Cobas Argos shows good correlation with the manual leukocyte differential on normal samples for lymphocytes and neutrophils; acceptable correlation for eosinophils; and not very good correlation for basophils and monocyte populations. Concerning the monocytes, since the ranges of the data for the two methods are different (0 to 6.5% for the manual differential and 2.5 to 11.3% for the Cobas Argos), statistical comparison between the two methods is not possible. For basophils, the very low number of this cell usually found skews statistical calculations.

The correlation between the two methods is also good on abnormal samples. Concerning the monocytes, the correlation coefficient is slightly increased because of the 2 cases of monocytosis included. The higher values for this quantity make the statistical calculation more powerful.

During this study we found on the Cobas Argos slightly higher values for monocyte count versus the manual leukocyte differential. This is compatible with what other authors have found on other analysers (10).

For the determination of sensitivity and specificity of an haematological analyser versus the manual leukocyte differential, NCCLS, in the procedure named H20 T, recommends counting 800 cells on a total of 150 normal and 150 abnormal specimens.

This method requires a tremendous amount of work and several researchers have proposed their own procedures as an alternative to the H20 T.

Kohut suggests reading 100 cells on 4 different smears made from the same sample by 4 different technologists.

According to *Rümke* (11), it is not possible to rely on 100 cell counts due to the high CV's found between two counts.

Therefore, we have preferred to have 200 cells counted by two different experienced technicians both reading the same film. This technique has also already been used (12). The CV for automatic instruments is usually better than the CV on the manual differential; by counting more cells we made the two techniques more comparable in the sense of *Passing & Bablok*.

The predictive values, as well as the reasonably low number of false positive results given by the Cobas Argos 5 Diff, are equivalent to or better than those shown by other authors on other 5 differential analysers (13, 14).

Therefore, we have concluded that the Cobas Argos 5 Diff is a very good analyser for screening.

We are also pleased by the possibility of counting and differentiating leukocytes under 1.0×10^9/l. One disadvantage was the LCD screen. Instead of being really walk-away, the instrument forced the operator to stay in front of it. Another negative point was the temperature regulation of the mixing chamber, a problem that has since been solved.

In our opinion, the use of haemoglobin reagent may on occasion cause an environmental problem, which is a problem that must be solved. The sample tray must be adapted for all tube formats commercially available.

In conclusion, the Cobas Argos 5 Diff is a user friendly, fast and compact analyser, comparing very well with other analysers of same class. Microscopic analysis and morphological knowledge will, however, continue to be very important.

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